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The Effect of Gibberellins on Flowering in Roses

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Abstract. The gibberellins A_1, A_3, A_5, A_8, A_{19}, A_{20}, and A_{29} were identified in vegetative shoot tips of *Rosa canina* by comparing their mass spectra and Kovats retention indices with those of standards. Most wild roses have a short flowering season of 2–4 weeks in spring, whereas most modern cultivars flower recurrently. 'Félicité et Perpétue' is a short-season hybrid from a cross between a wild rose and a recurrent-flowering rose, whereas its sport, 'Little White Pet,' flowers recurrently. The concentrations of gibberellins (GAs) were measured in shoot apices of both cultivars. In March (before floral initiation in spring) the concentrations of GA_1 and GA_3 were respectively threefold and twofold higher in 'Félicité et Perpétue' than in 'Little White Pet.' In April (after floral initiation) the concentrations of both gibberellins were substantially greater than in March, and concentrations of GA_1 and GA_3 were, respectively, 17-fold and 12-fold greater in 'Félicité et Perpétue' than in 'Little White Pet.' It is postulated that, in 'Félicité et Perpétue,' floral initiation occurs when concentrations of GAs are low and is inhibited when concentrations of GAs are high, whereas in 'Little White Pet' concentrations of GAs remain at permissive levels throughout the growing season. Applications of GA_1 and GA_3 to axillary shoots in March inhibited floral development in 'Félicité et Perpétue' but not in 'Little White Pet.' This suggests that the combined concentration of exogenous and endogenous gibberellins might have been raised to inhibitory levels in the former but not in the latter cultivar.

Key Words. Gibberellins—Recurrent-flowering—*Rosa*—Seasonal-flowering—GC-MS

Abbreviations: CVC, centrifugal vacuum concentrator; GA, gibberellin; GC-MS, gas chromatography-mass spectrometry; KRI, Kovats retention indices; SIM, selected ion monitoring; TLC, thin-layer chromatography.

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Most wild roses have a short flowering season of 2–4 weeks in spring. Modern cultivars flower recurrently, producing flowers throughout the growing season outside and throughout the year under glass. Their flowering is described as self-inductive because there is no evidence that floral initiation is environmentally regulated (Halevy 1972). The recurrent-flowering characteristic is determined by a recessive gene that arose as a mutant in *Rosa chinensis* (Hurst 1941, Thomas 1994). This gene was selected in China and used in rose-breeding as early as the Song Dynasty (AD 960–1279) (Ogisu 1996). Recurrent-flowering cultivars of *R. chinensis* and *R. chinensis* × *gigantea* that were introduced to Europe and America circa AD 1800 are the progenitors of our modern recurrent-flowering cultivars.

'Félicité et Perpétue' is a diploid hybrid (2n = 14) between *R. sempervirens* (short-season) and a pollen parent that is thought to be a Noisette rose (recurrent flowering) (Cairns (1993). 'Félicité et Perpétue' itself has a short flowering season, as would be expected of a hybrid between a short-season and a recurrent-flowering rose, but it gave rise to a recurrent-flowering sport, 'Little White Pet.' We presume that, in the origin of 'Little White Pet,' the wild-type allele of the recurrent-flowering gene mutated to a form that permits the recurrent-flowering character to be expressed. The stature of the recurrent-flowering sport is restricted because the elongation of vegetative axes is terminated repeatedly by the initiation of flowers. However, apart from their phenological differences and the consequential effects on habit, the two cultivars are similar in appearance and are suitable subjects for comparisons between short-season and recurrent-flowering roses.

In spring, garden-grown roses produce shoots from axillary buds on stems that were formed in the previous season. The terminal meristem of each axillary shoot initiates a number of leaf primordia that is characteristic of a particular genotype (Cockshull and Horridge 1977, Horridge and Cockshull 1974). Shoot growth is terminated by the inflorescence, and further growth involves

the development of axillary shoots on these stems. Vegetative growth then predominates in short-season roses, whereas the initiation of leaves followed by an inflorescence is repeated cyclically in recurrent-flowering roses.

Gibberellins (GAs), when applied exogenously, inhibit flowering in many woody angiosperms (Metzger 1987, Zimmerman et al. 1985), including the woody rosaceous species *Malus domestica* (Tromp 1982), *Prunus avium* (Bradley and Crane 1960, Facteau et al. 1988, Olivera and Browning 1993) and *P. cerasus* (Bukovac and Yuda, 1991). Abad Farooqi et al. (1994) showed that leaves of nonflowering shoots of *R. damascena* (short-season) contained higher concentrations of GA-like substances than flowering shoots. Furthermore, applications of GA_3 reduced the number of flowers per plant and the proportion of flowering plants. However, in a study of flower-bud atrophy in *R. hydrida* 'Baccara,' Zieslin, and Halevy (1976a) reported that concentrations of endogenous growth substances, including those with GA-like activity, were lower in leaves of nonflowering shoots than flowering shoots. Also, shoot blindness could be alleviated by spray applications of 100 mg L^{-1} GA_3 but, when the concentration was increased to 1000 mg L^{-1}, flowering was inhibited (Zieslin and Halevy 1976b).

In this investigation we have used gas chromatography-mass spectrometry (GC-MS) to identify unequivocally the GAs present in shoots of a wild specimen of *R. canina* and have used GC-MS selected ion monitoring (SIM) to quantify GAs in the short-season rose 'Félicité et Perpétue' and its recurrent-flowering sport 'Little White Pet.' Also, the effects of exogenously applied GAs were contrasted in these two varieties. The effect of exogenous GA_3 on flowering was also studied in *R. wichuraiana* (short-season) and on unrelated cultivar, 'Eyepaint' (recurrent-flowering).

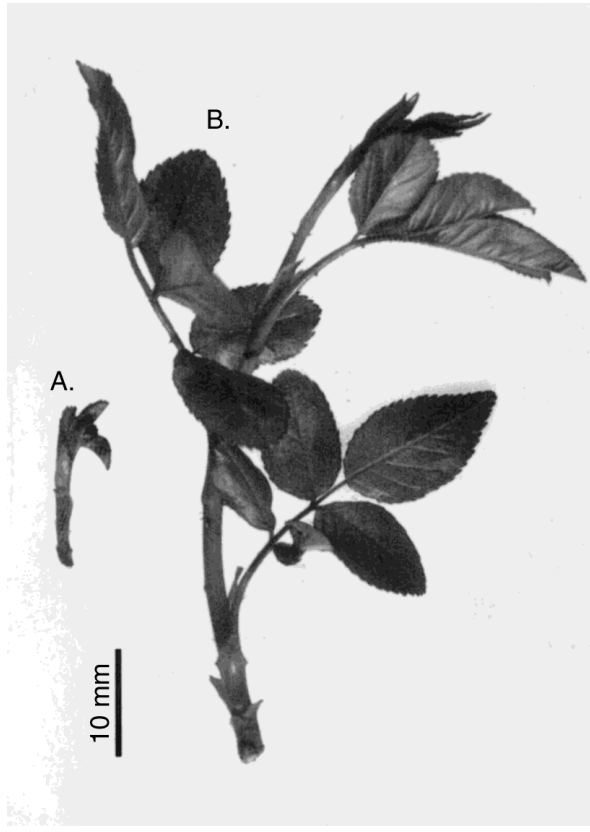

Fig. 1. Shoot tips of 'Félicité et Perpétue' *(A)* in March before floral initiation, when samples were taken for analysis of gibberellins and GA_1 and GA_3 were applied; *(B)* in April after floral initiation, when samples were taken for analysis of gibberellins.

Materials and Methods

Plant Material

Plants of 'Félicité et Perpétue,' 'Little White Pet," and "Eyepaint,' which were budded onto *R.* 'Laxa' rootstocks and self-rooted *R. wichuraiana* were maintained in a garden for 2 years before the start of the investigation. Plants of *R. canina* were growing wild near East Malling in Kent, UK.

In autumn, four shoots arising from the base of each of the three garden-grown plants of 'Félicité et Perpétue' and 'Little White Pet' were trained horizontally at a height of 200–400 mm above soil level in preparation for exogenous application of GA_1 the following spring. Plants of 'Félicité et Perpétue,' 'Little White Pet,', *R. wichuraiana*, and 'Eyepaint' were similarly prepared for applications of GA_3. After bud break in spring, an emergent shoot at the middle of each branch was designated for treatment and an adjacent shoot (at a distance of 35–45 mm) was designated as a control. Solutions (2 mg mL^{-1}) of GA_1 or GA_3 (Sigma, Poole, UK) in 70% v/v ethanol were prepared. Aliquots (2 μL) of GA_1 or GA_3 solution were applied to the tips of axillary shoots (Fig. 1*A*) on alternate days over a period of 2 weeks early in March. Aliquots (2 μL) of 70% v/v ethanol were applied to the control shoots. After flowering, the number of nodes, the internodal lengths, and the number of flowers per inflorescence were recorded on treatment and control shoots in early June.

For identification of GAs, shoot tips that included the expanding leaves of rapidly growing stems of *R. canina* were collected during early April and frozen immediately in liquid nitrogen before being stored at −70°C until hormone extraction.

Assays of the GA content of shoot tips of 'Félicité et Perpétue' and 'Little White Pet' were based on samples collected in early March shortly after bud break (Fig. 1A), early April when 3–4 leaves had expanded (Fig. 1B), and mid-September. Each shoot tip consisted of a terminal bud excised above an unexpanded leaf. Each sample consisted of 150 shoot tips taken from approximately 20 plants of each variety. Samples were frozen in liquid nitrogen and then stored at −70°C.

Extraction and Purification of Gibberellins

Frozen samples were homogenized in cold (4°C) 80% v/v methanol containing 20 mg L^{-1} butylated hydroxytoluene (5.0 mL g^{-1} FW) and stirred overnight at 4°C. To each sample, 0.50 KBq of [1,2-^{3}H]GA_1 (1406 GBq $mmol^{-1}$; Du Pont de Nemours GmbH, NEN Division, Dreiech, Germany) was added before homogenization to estimate recoveries. After filtration, the residue was re-extracted with 200 mL methanol for 4 h and refiltered. The filtrates were combined and metha-

nol was removed under reduced pressure on a rotary film evaporator at 30°C. An equal volume of pH 8.2 potassium phosphate (0.5 M) buffer was added to the aqueous residue, and the pH was adjusted to 8.0 with KOH (1 M) before freezing in liquid N_2. After thawing, the extract was centrifuged (33,000 × *g*; 15 min at 4°C), and the supernatant was decanted and added to a column (15 × 50 mm) of insoluble polyvinylpolypyrrolidone pre-equilibrated with pH 8.2 buffer. After loading, the column was washed with a further 15-mL pH 8.2 buffer, and the eluates were combined and adjusted to pH 2.5 with HCl (2 M) before being partitioned against ethyl acetate (3 × equal volumes). The combined organic phases were back extracted into 5% v/v sodium bicarbonate (3 × 1/5 volume); this was then acidified to pH 3.0 (2 M HCl) and partitioned against ethyl acetate (3 × equal volumes), and the combined organic layer was then washed with pH 3.0 water (3 × 10 mL) and reduced to dryness using the rotary film evaporator. The extract was dissolved in 5 mL water, the pH was adjusted to 8.0 with KOH (1 M), and the extract was then added to a column (15 × 100 mm) of QAE Sephadex A-25 (Pharmacia, Central Milton Keynes, UK), pre-equilibrated with sodium formate (0.5 M) and washed with formic acid (0.2 M) and water (pH 8.0). After loading, the column was washed with pH 8 water (60 mL) and GAs were eluted with 0.2 M formic acid (80 mL). The eluate was fed directly through two pre-equilibrated C_{18} Sep-Pak cartridges (Waters Associates, Watford, UK) in series; after washing with 5.0 mL water (pH 3.0); GAs were eluted with 80% v/v methanol (20 mL), which was then evaporated to dryness *in vacuo*.

GAs were purified further by reverse phase HPLC (Hewlett Packard series 1050, Winnersh, UK) using a 4.6 mm ID × 250-mm column containing Hypersil ODS (Hichrom Ltd., Reading, UK). The column was eluted at a flow rate of 1.0 mL min^{-1} with 10% v/v methanol for 5 min, followed by a linear gradient to 100% methanol over 45 min (solvents contained 50 μL L^{-1} acetic acid). Samples were dissolved in 10% v/v methanol (200 μL) and injected into the column using a 500 μL loop. Fifty 1-mL fractions were collected, and aliquots ($^1/_5$) were removed for bioassay using the lettuce (*Lactuca sativa* 'Arctic King') hypocotyl test (Frankland and Wareing 1960); the remaining fractions were taken to dryness using a centrifugal vacuum concentrator (CVC). The extracts were redissolved in a small volume of methanol. Fractions were then combined as necessary and methylated with excess ethereal diazomethane before the methylated extracts were taken to dryness under a stream of O_2-free N_2 redissolved in dry ethyl acetate (50 μL) and passed through a pre-equilibrated NH_2 Supelclean SPE Tube (Supelco Inc., Poole, UK). More ethyl acetate (200 μL) was then passed through and the combined eluates were taken to dryness in the CVC. The MeTMSi derivatives were prepared by adding 25 μL Tri-Sil BSA (Pierce & Warriner, Chester, UK), heating to 100°C for 5 min, evaporating to dryness, and redissolving in 5 μL BSTFA (Pierce and Warriner, Chester, UK) for GC-MS or fractions were purified further by thin-layer chromatography (TLC). For TLC, the combined ethyl acetate eluates were evaporated to dryness (CVC) and redissolved in 150 μL of dichloromethane and applied in a narrow band to 20 × 20 cm aluminium-backed silica gel coated (0.2 mm) plates (E. Merck, Darmstadt, Germany). Methylated GA standards were applied within a scored zone close to each vertical outside edge of the plate, and the plate was developed using chloroform/methanol (9:1 v/v) as the solvent system. After development, vertical strips enclosing the GA standards were cut from the plate, sprayed with sulfuric acid–ethanol (1:20 v/v), heated at 110°C for 10 min, and GAs detected under UV light at 254 nm. Silica gel in broad zones on the plate corresponding to the Rf of the standards was removed and packed into cotton-plugged Pasteur pipettes and eluted with 750 μL of ethanol. The ethanol was taken to dryness, and the MeTMSI derivatives were prepared for GC-MS as described previously.

For quantitation of GAs in shoot apices by GC-MS-SIM, the procedures described previously were used, but 67 ng of [$^2H_2$]- gibberellins A_1, A_3, A_8, A_{19}, A_{20}, A_{29} (obtained from Prof. L. N. Mander, Australian National University, Canberra, Australia) were added to each sample after the homogenization step, and these samples were not subjected to bioassay.

Recoveries of [1,2-^{3}H]GA_1 after these procedures were 50–60 %.

Capillary Column GC-MS

Extracts were analyzed using a TRIO- 1 (ThermoQuest, Manchester, UK) GC-MS system. The CP-SIL 5 CB-MS (Chromopack, London, UK) capillary column (25 m long × 0.25 mm ID) was coupled directly to the ion source with an interface temperature of 275°C, and the He carrier gas inlet was programmed to maintain a linear velocity of 400 mm sec^{-1}.

Derivatized extracts (MeTMSi, 1 μL) were injected (injector temperature of 270°C) at an oven temperature of 90°C with the injection splitter (50:1) closed. After 1 min, the splitter was opened and 1 min later the oven temperature was increased at 20°C min^{-1} to 220°C and then at 4°C min^{-1} to 290°C. Mass spectra were acquired by the VG Lab-Base (ThermoQuest, Manchester, UK) data system after 14 min, scanning from 50–650 amu at 0.9 s mass $decade^{-1}$. The electron energy was 70 eV and the source temperature 200°C. Kovats retention indices (KRI) for standard and endogenous GAs were measured using *n*-alkanes (Gaskin et al. 1971).

For quantitation by GC-MS-SIM, the data system was set to monitor ion clusters of m/z as follows: GA_1: 506, 507, 508, 509, 510, 511, 512; GA_3: 504, 505, 506, 507, 508, 509, 510; GA_8: 594, 595, 596, 597, 598, 599, 600; GA_{19}: 434, 435, 436, 437, 438, 439, 440; GA_{20}: 418, 419, 420, 421, 422, 423; and GA_{29}: 506, 507, 508, 509, 510, 511, 512. We did not quantify GA_5 as the ion m/z 418 of MeTMSi [$^2H_2$]-GA_5 interfered with the M^+ ion of MeTMSi GA_{20} under the GC conditions used. A GENSTAT program (Payne et al. 1987) was used to calculate, by nonlinear least-squares curve fitting of the normalized responses, the isotopic enrichment of the endogenous GAs by their deuteriated internal standards and, by means of an isotopic dilution equation, the amounts of the endogenous GAs present in each extract, as described by Gaskin and MacMillan (1991).

Statistics

For each variety, measurements of the amounts of GAs were based on three replicates derived from pooled samples of 150 shoot tips. These were log transformed to achieve homoscedasticity of variances and the significances of differences between means were tested by the Student's *t* test using pooled variances.

Results

After fractionation of extracts by reverse-phase HPLC, GA-like activity was detected by bioassay, in the acidic ethyl acetate extract, in fractions 22–25, 30–32 and 34–36 (data not shown). Groups of 2–4 of the biologically active or other HPLC fractions were combined as appropriate for full-scan GC-MS or SIM and for further purification of the methyl esters by TLC as described by Taylor et al. (1994). The TLC purification step was found to be necessary to eliminate ions from co-eluting compounds that were contaminating mass spectra, especially those of GA_1, GA_5 and GA_{20}, obtained after

Table 1. Comparison of Kovats retention indices and relative intensities of characteristic ions for MeTMSi derivatives of gibberellins in shoot tips of *R. canina* with those of standard compounds (or literature values [Gaskin and MacMillan, 1991] for standard compounds)

Identified gibberellin	Kovats retention index	Diagnostic ions (m/z) with abundance in reference and sample									HPLC fraction
GA_8		Ion	594 (M^+)	579	535	448	379	375	238	207	
	2818	Lit. reference	100	6	7	14	7	6	14	40	
	2830	Sample	100	5	7	25	18	15	29	90	17–18
GA_{29}		Ion	506 (M^+)	491	447	375	303	281	235	207	
	2684	Lit. reference	100	13	8	16	22	5	13	38	
	2698	Sample	100	5	5	30	9	9	16	13	18–19
GA_3		Ion	504 (M^+)	489	475	445	387	370	355	208	
	2713	Standard	100	11	15	19	14	26	19	94	
	2712	Sample	100	11	17	16	16	22	23	95	22–23
GA_1		Ion	506 (M^+)	491	448	377	376	313	235	207	
	2693	Standard	100	11	20	24	27	33	21	45	
	2692	Sample	100	11	25	22	22	18	19	72	23–25
GA_{20}		Ion	418 (M^+)	403	389	375	359	301	235	207	
	2517	Standard	100	18	7	71	21	27	18	96	
	2517	Sample	100	17	8	84	25	26	10	44	30–31
GA_5		Ion	416 (M^+)	401	385	372	257	343	299	207	
	2513	Standard	100	20	2	5	19	16	43	40	
	2512	Sample	100	24	4	6	23	17	53	45	31–32
GA_{19}		Ion	462 (M^+)	434	402	375	374	345	285	208	
	2627	Standard	4	96	52	84	96	45	38	100	
	2629	Sample	4	91	38	72	100	44	33	85	34–36

HPLC. A total of seven 13-hydroxylated GAs were identified by a comparison of full-scan mass spectra and KRI with those data for protio GA standards or literature values (Gaskin and MacMillan 1991); KRI and data derived from full-scan mass spectra are presented (Table 1). Fractions 23–25 were found after TLC to contain endogenous GA_1; fractions 22–23, GA_3; fractions 30–31, GA_{20}; fractions 31–32, GA_5; and fractions 34–36, GA_{19}. GA_8 and GA_{29} were identified, following TLC of fractions 17–19, from a comparison with published mass spectra and KRI (Gaskin and MacMillan 1991) and by comparison with the KRI (2829 and 2697, respectively) we obtained for MeTMSi-[17-$^2H_2$]GA_8 and [17-$^2H_2$]GA_{29} (the KRIs of [17-$^2H_2$]GAs and their corresponding protio GAs characteristically differ by a KRI of 1 under our conditions). The KRIs we obtained for protio GA_3, GA_1, GA_{20}, GA_5, and GA_{19} are greater by 21, 23, 35, 36, and 31, respectively, than the values published by Gaskin and MacMillan (1991).

The predominant GAs in all shoot-tip samples were GA_1, GA_3, and GA_{19} (Fig. 2). GA_{19} has been shown to be an inactive precursor of the biologically active GAs, GA_1 and GA_3 (for reviews, see Graebe 1987, MacMillan 1997). Other GAs that were identified in lower concentrations, GA_8, GA_{20}, and GA_{29}, are either intermediary metabolites or inactive end products (for reviews, see Graebe 1987, MacMillan 1997). In 'Félicité et Perpétue,' GA_3 was present at concentrations of 13.7 ng.g^{-1} FW in March, 285.7 ng g^{-1} FW in April, and 12 ng g^{-1} in September (Fig. 2). In 'Little White Pet,' GA_3 was present in much smaller amounts: 7.3 ng g^{-1} FW in March, 22.9 ng g^{-1} FW in April, and 2 ng g^{-1} in September (Fig. 2). Concentrations of GA_1 were lower than GA_3 at all sampling times and were significantly lower ($p < 0.001$) in 'Little White Pet' than in 'Félicité et Perpétue' in March and April (Fig. 2). The source of differences in the concentrations of gibberellins in the two cultivars is likely to be the scion because both cultivars were budded onto 'Laxa' rootstock.

Shoot tips of 'Félicité et Perpétue' that were treated with GA_3 in early March produced significantly fewer flowers per shoot (0.3 ± 0.25) than the controls (15.6 ± 2.8) (Table 2, Fig. 3). In contrast, GA_3-treated and control shoot tips of 'Little White Pet' did not differ significantly (Table 2, Fig. 3). Likewise, GA_1 induced a significant reduction in the mean number of flowers per inflorescence in 'Félicité et Perpétue' but not in 'Little White Pet' (Table 3). In 'Félicité et Perpétue' and 'Little White Pet' shoots treated with either GA_1 or GA_3 had significantly longer internodes than the untreated controls. The number of nodes below the inflorescence did not differ significantly from the controls in treatments with GA_1 or GA_3, indicating that the vegetative nodes were preformed in the bud at the time that treatments commenced (Tables 2 and 3). This was confirmed by counting the leaves in similarly sized buds at the time of the first applications.

Applications of GA_3 to shoot tips completely sup-

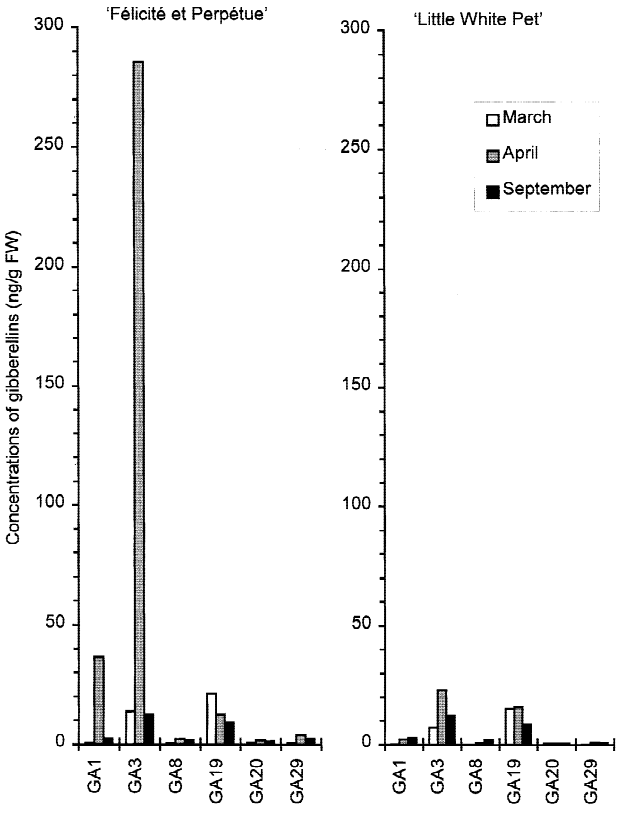

Fig. 2. Concentrations of gibberellins in shoot tips of 'Félicité et Perpétue' and 'Little White Pet' before (March) and after (April) floral initiation and in September.

pressed flowering in *R. wichuraiana* but did not significantly affect the number of flowers per inflorescence in 'Eyepaint' or the number of nodes below the inflorescence (Table 4).

Discussion

Previously, GA-like activity was studied in leaves of the rose 'Baccara' by paper chromatography and bioassay (Zieslin and Halevy 1976a), GA-like substances were studied in vegetative tissues of *R. damascena* by TLC and bioassay (Abad Farooqi et al, 1994), and 13-hydroxy GAs were studied in reproductive organs of *R. hybrida* by HPLC and immunoassay (Bianco et al. 1991). However, this work reports the first identification of GAs in vegetative tissues of roses. The GAs that were identified are members of the early 13-hydroxylation pathway. This is a major biosynthetic pathway operating in the vegetative tissues of many plants. It yields GA_1 as an active product, which is known to be important in the control of shoot growth (for reviews, see Graebe 1987, MacMillan 1997). In some species, GA_3 is an additional active product (Albone et al. 1990, Fujioka et al. 1990).

Table 2. Phenological parameters of control and GA_3-treated axillary shoots of 'Félicité et Perpétue' and 'Little White Pet'

Genotype/treatment	No. of flowers per inflorescence	Mean internode length (mm)[a]	No. of nodes below inflorescence[a]
'Félicité et Perpétue'			
Control	15.6 ± 2.82	31.6 ± 1.73	6.3 ± 0.37
GA_3 treatment	0.3 ± 0.25	52.2 ± 2.21	6.9 ± 0.59
t test probability	***	***	NS
Degrees of freedom	14	13	13
'Little White Pet'			
Control	11.4 ± 1.94	34.4 ± 2.39	6.0 ± 0.38
GA_3 treatment	12.0 ± 3.77	54.6 ± 3.10	6.0 ± 0.38
t test probability	NS	***	NS
Degrees of freedom	14	14	14

Data are means ± SEM, NS, not significant at $p = 0.05$, *** significant at $p = 0.001$.

[a] One treated shoot of 'Félicité et Perpétue' did not have branches marking the start of the "inflorescence." Therefore, values could not be assigned to "number of nodes below inflorescence" or "mean internode length."

There is evidence for the occurrence of GAs of the early 13-hydroxylation pathway in vegetative tissues of other species in the Rosaceae including apple (Koshioka et al. 1985, Saavedra et al. 1989), cherry (P.S. Blake and G. Browning, unpublished), and strawberry (Taylor et al. 1994).

The short-season cultivar 'Félicité et Perpétue' differed from its recurrent-flowering sport, 'Little White Pet,' in the concentrations of gibberellins that were present in March and April. In March, before floral initiation, the concentrations of GA_1 and GA_3 were, respectively, threefold and twofold greater in 'Félicité et Perpétue' than in 'Little White Pet.' In April, after floral initiation, the concentrations of GA_1 and GA_3 were, respectively, 17-fold and 12-fold greater in 'Félicité et Perpétue' than in 'Little White Pet.' The simplest interpretation of the origin of 'Little White Pet' as a sport of 'Félicité et Perpétue' is that it resulted from the mutation of a gene involved in the GA biosynthetic pathway and that its ability to flower recurrently is associated with a consequential reduction in the concentration of some GAs. It is postulated that floral induction in 'Félicité et Perpétue' is permitted shortly after bud break in spring when concentrations of GAs are low and is inhibited by the subsequently higher levels of GAs. However, low concentrations of GAs permit floral induction in 'Little White Pet' throughout the growing season. The higher gibberellin-like activity in leaves of nonflowering compared with flowering plants of *R. damascena* (Abad Farooqi et al. 1994) also provides evidence of inhibition of floral initiation by high concentrations of GAs.

Applications of GA_1 and GA_3 to shoot tips in March were found to suppress floral initiation in 'Félicité et

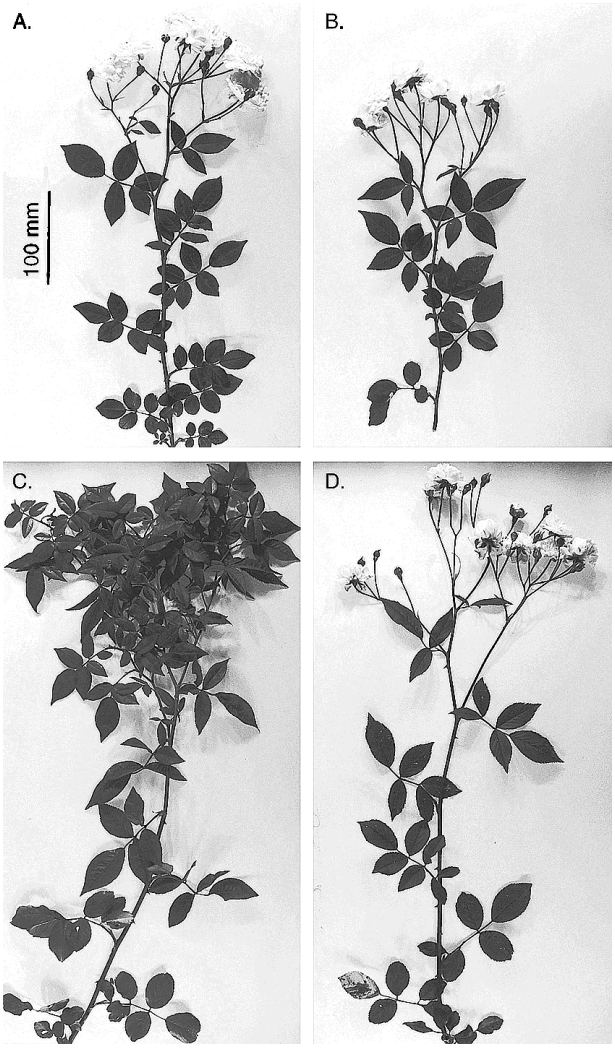

Fig. 3. GA_3-treated and control shoots of 'Félicité et Perpétue' and 'Little White Pet' in June. *(A)* Control of 'Félicité et Perpétue;' *(B)* control of 'Little White Pet;' *(C)* GA_3-treated shoots of 'Félicité et Perpétue;' *(D)* GA_3-treated shoots of 'Little White Pet.'

Table 3. Phenological parameters of control and GA_1-treated axillary shoots of 'Félicité et Perpétue' and 'Little White Pet'

Genotype/treatment	No. of flowers per inflorescence	Mean internode length (mm)[a]	No. of nodes below inflorescence[a]
'Félicité et Perpétue'			
Control	13.3 ± 2.21	30.9 ± 3.67	7.1 ± 0.55
GA_1 treatment	0.1 ± 0.13	45.0 ± 3.05	7.6 ± 0.61
t test probability	***	*	NS
Degrees of freedom	14	13	13
'Little White Pet'			
Control	13.3 ± 3.02	27.6 ± 1.99	5.5 ± 0.27
GA_1 treatment	13.5 ± 3.13	46.4 ± 2.55	6.1 ± 0.30
t test probability	NS	***	***
Degrees of freedom	14	14	14

Data are means ± SEM, NS, not significant at $p = 0.05$, * significant at $p = 0.05$, *** significant at $p = 0.001$.

[a] One treated shoot of 'Félicité et Perpétue' did not have branches marking the start of the "inflorescence." Therefore, values could not be assigned to "number of nodes below inflorescence" or "mean internode length."

Table 4. Phenological parameters of control and GA_3-treated axillary shoots of *Rosa wichuraiana* and 'Eyepaint'

Genotype/treatment	No. of flowers per inflorescence	Mean internode length (mm)[a]	No. of nodes below inflorescence[a]
Rosa wichuraiana			
Control	25.0 ± 8.00	34.8 ± 4.88	10.3 ± 0.71
GA_3 treatment	0	—	—
t test probability	***	—	—
Degrees of freedom	14	—	—
'Eyepaint'			
Control	15.9 ± 8.56	32.9 ± 5.60	9.8 ± 1.67
GA_3 treatment	20.8 ± 13.56	59.5 ± 11.57	9.9 ± 5.30
t test probability	NS	***	NS
Degrees of freedom	14	14	14

Data are means ± SEM, NS, not significant at $p = 0.05$, *** significant at $p = 0.001$.

[a] GA_3-treated shoots of *Rosa wichuraiana* were unbranched, without flowers. Therefore, values could not be assigned to "number of nodes below inflorescence" or "mean internode length."

Perpétue' but not in 'Little White Pet.' Applications of GA_3 also resulted in suppression of flowering in the short-season roses, *R. wichuraiana* (this investigation) and *R. damascena* (Abad Farooqi et al. 1994) but not in the recurrent-flowering rose 'Eyepaint' (this investigation). One interpretation of this is that the combined concentrations of exogenous and endogenous gibberellins were sufficiently high to suppress flowering in the short-season roses but not in the recurrent-flowering roses. An alternative interpretation, that short-season and recurrent-flowering roses differ in their sensitivity to GAs, is less likely because it implies that two mutations were involved in the origin of 'Little White Pet'. Zieslin and Halevy (1976b) showed that flowering could be suppressed in the recurrent-flowering rose 'Baccara' by spray applications of GA_3 at sufficiently high concentration (1000 mg L^{-1}).

The fall in the concentrations of gibberellins in 'Félicité et Perpétue' and 'Little White Pet' in late summer may be attributable to a reduction in meristematic activity at that time of year. No second flowering of 'Félicité et Perpétue' occurs in the UK, but some other short-season genotypes do have a late flush of flowers in autumn. This late flowering may be initiated in summer after the concentrations of gibberellins fall to a level that permits flowering. Further research is needed to establish

how rapidly floral initiation might occur after concentrations fall to permissive levels. It would also be interesting to investigate the possibility that growth retardants that inhibit GA biosynthesis (for review see Rademacher 1989) might induce out-of-season flowering in short-season roses.

Short-season and recurrent-flowering roses have clearly contrasting phenologies with simple dominant/recessive genetic determination. Thus they provide unique opportunities for physiologic and molecular studies of the control of flowering in woody plants. The hypothesis that floral development is permitted only when concentrations of GAs are low is suggested as a subject worthy of further investigation. Abad Farooqi et al. (1994) showed that applications of cytokinins could increase the number of flowers produced on *R. damascena* (short season) and that there may be interactive effects of gibberellins and cytokinins.

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